Remarks

The claimed invention

The previously pending claims have been replaced by new claims drawn to particular embodiments of the invention. In particular, certain of the claims are drawn to transgenic non-human mammals, wherein mammary gland secretory cells express and secrete an active lysostaphin protein, wherein the secreted lysostaphin is present in detectable quantities in milk produced by the mammal.

Information Disclosure Statement

The Examiner indicated that the information disclosure statement filed July 9, 2002, fails to comply with 37 C.F.R. 1.98(a)(2), which requires a legible copies of the references, and also fails to comply with 37 C.F.R. 1.98(a)(1), which requires a list of all references submitted for consideration by the Office. Applicants would like to point out that the references marked on the PTO-1449 were provided in a previous application to which the instant application claims priority, i.e., U.S.S.N. 09/337,079, as indicated in Part IV.A.2 of the Information Disclosure Statement that was filed July 3, 2002, a copy of which is enclosed. Accordingly, it is submitted that copies of these references need not be provided. In addition, a copy of the initialed PTO-1449 from the parent case is enclosed, indicating that the references were indeed supplied. Applicants herewith submit a copy of the PTO-1449 that was submitted previously in the instant case and respectfully request that the Examiner consider all of the listed references.

Priority

The Examiner has indicated that Applicant has not complied with the requirement that the first sentence of the application must contain a specific reference to any application to which the benefit of priority is claimed whereas such priority is claimed in the Declaration. The specification has been amended accordingly to include a priority claim to provisional application 60/090,175, filed June 22, 1998, consistent with the Declaration, which correctly stated the priority claimed by the inventors.

Specification

The Examiner has objected to the disclosure in that the first line of the specification does not include a claim of priority to provisional application 60/090,667 while the Declaration does contain such a claim. As indicated above, the specification is amended herein to include the priority claim.

Claims

Claims 32-44, 46, 47 and 49 are objected to on the ground that they encompass embodiments broader than the elected invention. These claims have been cancelled. The new claims encompass only the elected invention. In particular, they are drawn to embodiments of the invention in which the transgenic mammal comprises a lysostaphin gene.

Rejections under 35 U.S.C. § 112

Claims 47 and 49 stand rejected under 35 U.S.C. § 112, first paragraph, on the ground that the specification does not enable the claims across their full scope. The Examiner has previously indicated that the claims are enabled to the extent that they encompass a transgenic non-human mammal that expresses an active anti-microbial protein (such as lysostaphin) in mammary secretory cells such that the active anti-microbial protein is detectable in milk produced by the non-human transgenic mammal. See Office Action dated 2/14/2004 in the instant application and Office Action dated 09/29/2001 in U.S.S.N. 09/337,079, of which the instant application is a Continuation-in-Part. However, the Examiner asserts that the specification does not reasonably provide enablement for a transgenic non-human mammal comprising a transgene encoding any anti-microbial protein in any tissue and/or cell of said mammal.

Claims 47 and 49 have been cancelled, and certain aspects of the subject matter encompassed within these claims is now found in new independent claims 65, 69, and 75. Thus the enablement of the present claims is discussed herein as though the rejection of claims 47 and 49 had been applied to the instant claims.

Claim 65 is drawn to a non-human transgenic mammal whose genome contain a transgene, wherein the transgene comprises, in operable association: (i) a mammary gland specific promoter; (ii) a DNA portion having a sequence that encodes a secretion signal sequence

functional in mammary gland secretory cells; and (iii) a DNA portion having a sequence that encodes an active lysostaphin protein, wherein a milk-producing non-human transgenic mammal having the genotype of the non-human transgenic mammal expresses the transgene in mammary gland secretory cells such that the active lysostaphin protein is detectable in milk produced by the transgenic non-human mammal. Claim 70 recites particular promoters that can be used in the transgene. Support for these claims is found throughout the specification and in the cancelled claims. See, e.g., p. 32, lines 13-17 in the specification (paragraph 125) stating that various promoters may be used to direct expression of the lysostaphin protein in the mammary gland and reciting particular promoters. See also p. 12, lines 20-25 in the specification (paragraph 56) reciting various mammalian signal peptides that direct secretion of lysostaphin in mammalian cells, including sequences previously shown to direct secretion from mammary gland secretory cells. See also Example 4, pp. 32-36 (paragraphs 125-139), which describe secretion of active lysostaphin from mammary gland secretory cells in milk-producing transgenic mammals of the invention and the resulting resistance to staphylococcal mastitis. As indicated above, the Examiner has already indicated that the invention is enabled with respect to a transgenic non-human mammal that expresses an active anti-microbial protein (such as lysostaphin) in mammary secretory cells such that the active anti-microbial protein is detectable in milk produced by the non-human transgenic mammal.

Claim 75 is identical to claim 65 but does not require a mammary gland specific promoter but rather a promoter functional in mammary gland secretory cells. Support for this claim, and claims 76-78, which depend on claim 75, is found throughout the specification and cancelled claims, as described above for claim 65, and elsewhere. In particular, the specification describes a number of different non tissue-specific promoters that could be used. See, e.g., (p. 11, lines 25-27 (paragraph 52) mentioning the CMV promoter, RSV promoter, and human EF1 α subunit promoters as promoters that can be used if it is not desired to restrict expression to mammary cells. The specification also describes constructs comprising these promoters, from which transgenic mammals can be made. For example, the specification discusses the CMV promoter, which is known to function in a wide range of mammalian cell types and describes its use to drive lysostaphin expression in COS-7 cells, which are monkey kidney cells. See, e.g., pp. 22-26 of the specification (paragraphs 96-100). The RSV promoter was used in adenovirus constructs that directed expression of β-galactosidase by mammary gland secretory cells of goats

infected with the adenovirus. See, e.g., p. 26, line 11 – p. 28, line 15 (paragraphs 102-111). The Av1LacZ4 construct employed in these experiments includes an RSV promoter (Smith, et al., Nat. Genet. 5:397-402, 1993). Similar adenovirus constructs also directed expression and secretion of detectable amounts of active lysostaphin by mammary gland secretory cells of goats infected with the adenovirus. See p. 28, line 16 – p. 30, line 4 (paragraphs 112-116). These constructs contained a CMV promoter operatively linked to a DNA portion encoding active lysostaphin, and a mammalian signal peptide. Thus it is evident that transgenes in which either of these promoters, or other promoters functional in mammary gland secretory cells and also functional in other cell types drive expression of an operatively linked DNA sequence encoding lysostaphin at levels detectable in milk of a mammal whose genome comprises the transgene.

The Examiner has asserted that delivery of lysostaphin by repeated administration and oral administration causes specific serum antibody titers to lysostaphin and that in order to successfully produce a transgenic animal expression of the transgene must be such that it does not induce an immune response. Applicants submit that they are not required to prove the non-existence of purely hypothetical problems such as a possible immune response in the absence of any evidence to suggest that such a response might exist or, if such evidence existed, evidence that the immune response would preclude enablement of the claims.

The Examiner also asserts that the specification fails to teach what to do with transgene expression of an anti-microbial protein such as lysostaphin if expressed in other tissues. As mentioned above, Applicants are not required to prove the non-existence of, or to present a solution to, purely hypothetical problems. Expression of lysostaphin in other tissues is irrelevant to the enablement of the claims.

Applicants therefore submit that the new claims, and claims dependent therefrom, are fully enabled. The Examiner indicated that the specification was enabling for a transgenic non-human mammal whose somatic and germ cells contain a transgene, wherein said transgene comprises a mammary gland specific promoter; a mammary gland specific enhancer; a DNA sequence encoding a secretion signal sequence functional in mammary gland secretory cells; and a DNA sequence encoding an active anti-microbial protein. Independent claim 65 includes each of these elements except for the mammary gland specific enhancer. Independent claim 70 similarly includes these elements and recites particular promoters well known to direct expression in mammary gland secretory cells. Independent claim 75 is identical to claim 65 but

requires a promoter functional in mammary gland secretory cells rather than a mammary gland specific promoter.

With respect to the fact that these claims do not require that the transgene comprises a mammary gland specific enhancer, Applicants submit that the Examiner has not provided any evidence that would indicate that an enhancer of any type, let alone a mammary gland specific enhancer, is necessary for enablement of the claims. While an enhancer may direct higher level expression than would otherwise be the case, enablement of the claims does not require that the transgene directs expression at such levels. The presence of an enhancer may result in production of foreign proteins in milk at mg/ml levels. However, the inventors showed that much lower levels of lysostaphin, e.g. 75 μg/ml are sufficient to confer substantial resistance to staphylococcal infection. See p. 34, lines 10-11 (paragraph 133), describing a transgenic mouse line that produced 75 μg/ml lysostaphin in its milk, and p. 35, lines 16-25 (paragraph 136), describing the substantial resistance to staphylococcal infection exhibited by these mice. Such levels are routinely achieved in the mammary glands of transgenic mammals using constructs that lack mammary gland specific enhancers.

Applicants also draw the Examiner's attention to the fact that adenovirus constructs in which expression of a DNA sequence encoding β-galactosidase or active lysostaphin was driven by a CMV promoter resulted in secretion of the respective proteins by mammary gland cells when the adenovirus construct was introduced into goat mammary glands, as described above. In particular, the measured levels of lysostaphin in mammary gland secretions were 860 ng/ml and 1100 ng/ml in two individual glands, i.e., approximately 1 mg/ml. No mammary gland specific enhancer was present in the adenoviruses, thereby demonstrating that such an enhancer is not needed for enablement of the claims.

In summary, Applicants submit that abundant evidence exists to demonstrate the enablement of the presently claimed non-human transgenic mammals, and the Examiner has not provided any specific evidence to the contrary. Withdrawal of the rejection is respectfully requested.

Claim 32 stands rejected under 35 U.S.C. § 112, first paragraph, as being indefinite. The Examiner maintains that the claim is vague and unclear as to what is being altered in the transgene. The Examiner also maintains that the claim requires sequences for expression but that

what sequences are considered sufficient is not clearly set forth in the claim or specification. Claim 32 has been cancelled. Applicants submit that the new claims are not indefinite. In particular, the claims recite the elements needed for expression and secretion, e.g., promoter, signal sequence, and DNA portion having a sequence encoding the active lysostaphin.

The new claims do not contain the term "altered gene", which the Examiner found vague and unclear. Claims 67, 68, 73, and 74 indicate that the portion of the transgene encoding lysostaphin is altered relative to naturally occurring lysostaphin. Applicants submit that it is clear that what is being altered is a nucleic acid in the coding portion of the gene, wherein the alteration disrupts a post-translational processing event that would otherwise take place within mammalian cells. The specification describes sites at which such post-translational processing occurs. See, e.g.., p. 13, lines 14-16 (paragraph 58), describing sequences that are recognized by the mammalian glycosylation machinery. Sites at which other forms of post-translational processing (e.g., phosphorylation, disulfide bond formation, etc.) occur are known in the art. Withdrawal of the rejection is respectfully requested.

Claim 46 stands rejected under 35 U.S.C. § 112, first paragraph, as being indefinite in that the claim appears to be redundant. Claim 46 has been canceled.

In conclusion, in view of the amendments and remarks presented herein, the application and pending claims comply with the requirements of 35 U.S.C. §112. Applicants therefore respectfully submit that the present case is in condition for allowance. A Notice to that effect is respectfully requested.

If, at any time, it appears that a phone discussion would be helpful in resolving any remaining issues, the undersigned would greatly appreciate the opportunity to discuss such issues at the Examiner's convenience. The undersigned can be contacted at (617) 248-5000 or (617) 248-5071 (direct dial).

A check to cover the fee for a three (3) month extension of time is enclosed. Please charge any additional fees associated with this filing, or apply any credits, to our Deposit Account No. 03-1721.

Respectfully submitted,

Monica R. Gerber
Registration Number 46,724

Date: August 13, 2004

Choate, Hall & Stewart
Exchange Place
53 State Street
Boston, MA 02109
(617) 248-5000

3734545_1.DOC